

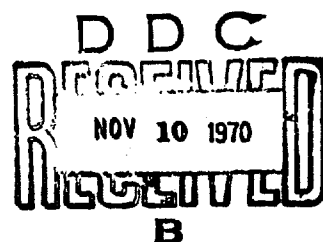
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An Improved Method for Preparation of Feces for Bomb Calorimetry¹

Hubert G. Lovelady and Emmett J. Stork



As a preliminary to bomb calorimetry, weighed, homogenized fecal slurries are prefrozen and lyophilized. Advantages of this method over the method of drying in a vacuum oven include: more moisture is removed from the sample initially, drying time is reduced from 48 h to 16 h, grinding is eliminated and objectionable odors are eliminated during sample processing.

THE CLASSICAL PROCEDURE for preparing feces for bomb calorimetry by the method of Atwater (*1*) has many inherent disadvantages. The temperature must be carefully controlled to avoid overheating and charring of the sample. Lengthy time periods (36 to 48 h) are required to ensure that the sample has been dried to a constant weight. Grinding is necessary to reduce the sample to a powder for pellet formation. An improved method has been developed for preparing human feces for calorimetry.

Method

Feces samples were obtained from human subjects on liquid diets over a specified time period. After the sample has been collected and weighed, distilled water, equal to the weight of the feces, is added. The feces-

water mixture is homogenized in a Waring Blendor for 3 to 5 min.

Approximately 20 g of the homogenized slurry are weighed and transferred to a 1-liter lyophilizing flask. The exact net weight is recorded. The feces mixture is prefrozen by swirling the flask in a container consisting of Dry Ice and acetone. Constant swirling is important in causing the mixture to freeze in layers, thus presenting a larger surface area for more efficient lyophilization. The frozen flask is attached to the freeze-dryer and lyophilized.

Drying time varies with sample size—e.g., 20 g of homogenized mixture requires approximately 8 to 16 h. When the mixture has dried completely, it is transferred to a weighing dish and the weight is determined to the nearest milligram. By knowing the starting weight and the dry weight, we can readily determine the moisture content: $[(B/A)(100) = C]$; $(100 - C = F)$, where A = grams of homogenized feces sample (wet), B = grams of dried feces, C = percent of dry weight, and F = percent moisture.

Pellets are prepared by weighing approximately 1 g of dried feces and placing this amount in the pellet press (Parr Instrument Co., Moline, Ill.). The finished pellet is accurately weighed and the weight is recorded as the weight of the sample going into the Parr oxygen bomb. From this point, bomb calorimetry of the sample proceeds as outlined in the Parr manual (*2*).

Calculations additional to those in the Parr manual are:

$$(0.5A/B)100 = C \quad (1)$$

$$(C)(D) = E \quad (2)$$

$$(E)(\text{kcal})(\text{observed from analysis}) = \text{kcal in}$$

$$\text{feces sample} \quad (3)$$

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Table 1. Comparison of Results for Calorimetry after Vacuum Oven-Drying and Freeze-Drying

Method	kcal/g (dry wt)	SD	kcal/g (wet wt)	SD
Vacuum oven, 60°C	5.505	±0.326	1.494	±0.089
Freeze-dry	5.270	±0.083	0.999	±0.17
	P < 0.7		P < 0.01	

where D = grams of total feces sample before homogenizing and E = grams of dry weight of total feces. The dilution factor in homogenizing is equal to 0.5 (see Equation 1).

Results and Discussion

The vacuum oven-drying method (60°C) was compared with the freeze-drying method. By the former method, 72.85 ± 0.0374 (SD) % moisture was removed, by the latter, $81.04 \pm 0.050\%$. The feces were from the same source. With the freeze-drying method total moisture removed is clearly greater, and the time required was only 16 h, as compared with 48 h required by the vacuum oven-drying method. There are other distinct advantages. Since measurement of the total energy of the sample is based on a rise in temperature at the point of combustion, freeze-drying during preparation eliminates any possibility of a rise in temperature that might lead to an error in calculating the total energy. Drying to a constant weight, which necessitates time-consuming multiple weighings, is not required. The

dried sample is in the form of a powder and requires no grinding. Unpleasant odors associated with the oven-drying method are avoided.

To better compare the old oven-drying method and the new freeze-drying method, caloric content of four specimens of the same sample of feces was measured by each method. The results were in good agreement (Table 1). The results shown in Table 1 for the wet weight in kilogram-calories per gram are calculated by multiplying the dry weight (kcal/g) by the percent remaining moisture (100% minus the percent moisture removed).

We feel that this method represents a distinct improvement over the original methods.

References

1. Atwater, W. O., Discussion of the terms digestibility, availability and fecal values. Twelfth Annual Report of Storrs Agricultural Experiment Station, Conn., 1899.
2. Parr Manual No. 120, Oxygen bomb calorimetry and oxygen bomb combustion methods. Parr Instrument Co., Moline, Ill., 1943.

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